Pharmacological Research, Vol. 46, No. 4, 2002
doi:10.1016/S1043-6618(02)00170-6, available online at http://www.idealibrary.com on IDEAL®

THE MEMORY EFFECT OF HETEROPOLYOXOTUNGSTATE (PM-19) PRETREATMENT ON INFECTION BY HERPES SIMPLEX VIRUS AT THE PENETRATION STAGE

KATSUAKI DAN[a,*], KIICHI MIYASHITA[a], YOSHIKO SETO[a], HARUHISA FUJITA[a] and TOSHIHIRO YAMASE[b]

[a]*Institute for Advanced Medical Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan,* [b]*Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, 227, Japan*

Accepted 4 July 2002

The keggin-type heteropolyoxotungstate $K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$ (PM-19) is a potent polyoxometalate (PM) inhibitor of the replication of herpes simplex virus (HSV). Pretreatment of Vero cells with PM-19 prior to HSV-2 infection enhanced the antiviral potency of PM-19 almost 10-fold compared with treatment of the cells only after infection. The pretreatment effect of PM-19 is called 'the memory effect'. The memory effect was reflected by inhibition of plaque formation and decrease of intracellular virus DNA quantity, and was strongest when PM-19 was present during the penetration stage of HSV-2 infection. The effect was maintained under conditions of fusion induced by polyethyleneglycol treatment. This suggests that PM-19 does not act at the fusion stage of infection. Using the infectious center assay method, it was clarified that a second round of infection was inhibited by about 30% in the presence of PM-19 at the penetration stage compared with the virus control in nontreated cells. The inhibition was enhanced to about 60% by PM-19 pretreatment prior to infection. This suggests that PM-19 pretreatment of the cells protects them against HSV-2 infection.

KEY WORDS: anti-HSV activity, PCR, penetration, polyoxometalate, viral genome synthesis.

INTRODUCTION

Some polyoxometalates (PMs) possess anti-viral or anti-tumor activity [1–13]. Keggin-type PMs are effective against herpes simplex virus (HSV). A keggin heteropolyoxotungstate ($K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$, PM-19) has been shown to be a potent PM inhibitor of the replication of HSV at a non-cytotoxic concentration [9]. The presence of PM-19 (over $10\,\mu g\,ml^{-1}$) completely inhibited the production of plaques and at $200\,\mu g\,ml^{-1}$ of PM-19 did not affect the growth of Vero cells. To clarify the mechanism of action of PM-19 against herpes simplex virus type 2 (HSV-2), we established a method of quantitating viral DNA by PCR [14]. Using this method, we demonstrated that PM-19 mainly inhibits the penetration of cells by virus [15].

In the study of antiviral chemotherapy, it is important to determine the condition of the cells treated with the antiviral agent. We confirmed that the PM-19 added to cells could be removed by washing the treated cells with phosphate-buffered saline (PBS) [15]. In this report, we describe the effects of PM-19 treatment prior to HSV-2 infection.

Pretreatment effects were evaluated by measuring of virus yields and cellular viral genome quantity after the infection. Treatment of Vero cells with PM-19 prior to the infection enhanced antiviral potency almost 10-fold compared with treatment of the cells only after infection.

MATERIALS AND METHODS

Cells and virus

Vero cells were cultured using 6-well multidishes of 35-mm diameter (Nalge Nunc International Inc., NY, USA) in Eagle's minimum essential medium (MEM; Nissui Pharmaceutical Co. Inc., Tokyo, Japan) supplemented with 5% fetal bovine serum (FBS) and nonessential amino acids. Vero cell monolayers were infected with HSV-2, strain 169, at 100 PFU per well after washing with PBS.

*Corresponding author. Institute for Advanced Medical Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan. E-mail: kdanmrw@sc.itc.keio.ac.jp

MEM supplemented with 1% FBS and nonessential amino acids was used as the experimental maintenance medium for cells infected with virus.

Anti-viral compounds

Polyoxotungstate ($K_7[PTi_2W_{10}O_{40}]\cdot 6H_2O$, PM-19) was synthesized by a method described previously [16]. Acyclovir (ACV) was provided by Wellcome Foundation Ltd. (UK) [17]. Each compound was dissolved in ultrapure water and stored at −80 °C until use. These stock solutions (PM-19, 10 mg ml^{-1} and ACV, 2 mg ml^{-1}) were diluted with the experimental cell maintenance medium for the experiments.

Chemicals

AmpliTaq polymerase and reaction agents for PCR, Takara Ex Taq, and cellular DNA extraction reagent, TRIzol® Reagent, were purchased from Takara Shuzou Co., Ltd., Kyoto, Japan and Gibco BRL, NY, USA, respectively. Other chemicals were purchased from Iwai Kagakuyakuhin Co., Ltd., Tokyo, Japan.

Oligonucleotide primers

Two 22-base oligonucleotides (5′-CAT CAC CGA CCC GGA GAG GGA C [positions in HSV-2; 3309–3330] and 5′-GGG CCA GGC GCT TGT TGG TGT A [positions 3400–3379]) deduced from the published sequence of the DNA polymerase gene-coding region from HSV were used [18]. This set of primers allows amplification of a 92-base pair fragment of the HSV DNA polymerase gene. These primers were synthesized by Sawady Technology Co., Ltd., Tokyo, Japan.

Estimation of memory effect

PM-19-pretreated or nontreated Vero cells were washed with PBS and then infected with HSV-2 at 100 PFU per well in the presence of PM-19 for various periods. After adsorption for 60 min at 4 °C, Vero cells were washed with PBS to remove unattached virus and were cultured in maintenance medium at 37 °C. For estimation of the memory effect, viral titers were determined by a plaque assay and infectious center assay. The cellular viral DNA quantity was also determined by a quantitative PCR method [14].

Promotion of virus-to-cell fusion by polyethyleneglycol

The infected cells were treated with polyethyleneglycol (PEG) according to the procedure of Sarmiento *et al.* [19]. Under the conditions, PEG did not induce cell-to-cell fusion in the Vero cultures, based on microscopic comparisons of the treated and untreated monolayers after fixation and staining.

Plaque assay

Plaque assays were done according to a method described previously [6]. Virus titers were expressed as plaque-forming units (PFU) and results were expressed as the number of plaques or as plaque inhibition (% of control).

Infectious center assay

Vero cells infected with HSV-2 were propagated at 10 h after the infection. The infected cells were overlaid on normal Vero cell monolayers and then incubated at 37 °C. PM-19 was added at various times. At 2 h after incubation, maintenance medium containing 2% methylcellose was overlaid, and infectious centers were counted 22 h later by neutral red staining.

Determination of cellular viral DNA quantity by PCR

Total cellular DNA was extracted from infected Vero cells at various times after infection. DNA was extracted using TRIzol® Reagent. DNA concentrations were calculated based on the A_{260} value for double-stranded DNA. The DNA samples were used directly for PCR amplification. Cellular viral DNA quantity was determined with a quantitative PCR method [14].

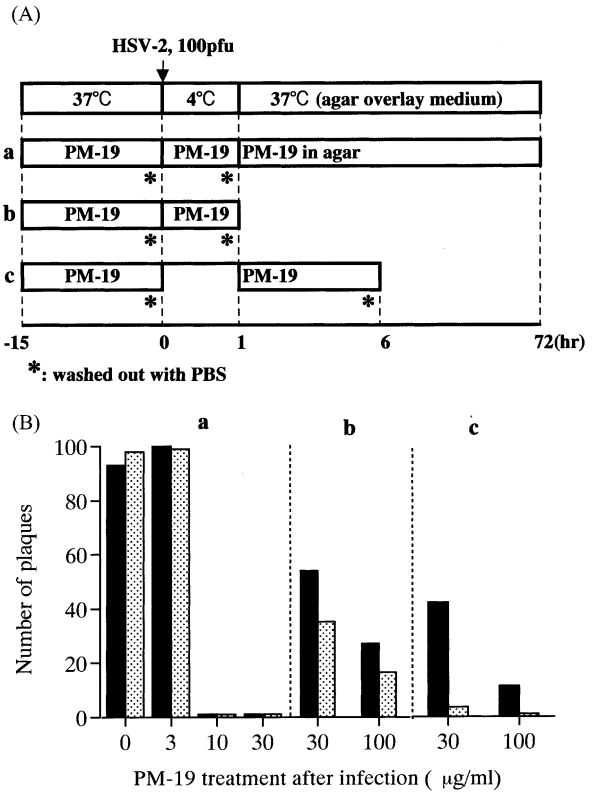

Fig. 1. (A) Experimental design. (B) Effect on HSV-2 replication of the time of exposure of Vero cells to various concentrations of polyoxotungstate (PM-19). Vero cell monolayers were treated with PM-19 (10 μg ml^{-1}) for 15 h prior to HSV-2 infection. After PM-19 was washed out with PBS, Vero cells were infected with the 169 strain of HSV-2 at 100 PFU/well and re-treated with several doses of PM-19 at various intervals. After the attachment of virus to cells for 60 min at 4 °C, the cells were washed with PBS and then overlaid with agar overlay medium. The results of the plaque assay were expressed as plaque numbers per well. Panel a, treated with PM-19 from 0 to 72 h; panel b, 0–1 h; panel c, 1–6 h. Black bars, not pretreated with PM-19; dotted bars, pretreated with PM-19.

RESULTS

Pretreatment effect of PM-19 on each stage of HSV-2 replication

To clarify whether PM-19-treated Vero cells had an altered response to HSV-2 infection, the plaque inhibition effect was determined with cells pretreated or nontreated with PM-19.

Pretreatment with PM-19 for 15 h prior to infection did not influence the plaque formation after HSV-2 infection (Fig. 1B, panel a; PM-19, 0 μg ml^{-1}). The pretreated Vero cells grew as well as nontreated cells (data not shown). Readdition of PM-19 at concentrations above 10 μg ml^{-1} from 0 to 72 h after infection caused a complete inhibition of plaque formation. However, no effect of pretreatment with PM-19 was found (Fig. 1B, panel a). In contrast, inhibition of viral attachment and penetration by PM-19 was enhanced in cells pretreated with PM-19 (Fig. 1B, panel b and c, respectively). These pretreatment effects of PM-19 were named 'the memory effect'.

Memory effect of PM-19 treatment prior to the infection on attachment and penetration in HSV-2 infection

The memory effect of PM-19 was expressed as a percentage of control plaques as shown in Figure 2. In the case of a 15-h pretreatment prior to infection, the memory effects of PM-19 on both the attachment and penetration stages of HSV-2 infection were increased with increasing concentrations of PM-19 during both pretreatment and readdition (Fig. 2A and B). There was no clear memory effect of PM-19 on attachment during the tested intervals of pretreatment with PM-19 (Fig. 2C), whereas the memory effect of PM-19 on penetration was shown clearly in cells pretreated with PM-19 for 1 h prior to infection (Fig. 2D). The maximal memory effect was observed when PM-19 was present at 10 μg ml^{-1} for 1 h before infection, and then again present during the penetration stage at 100 μg ml^{-1}. The inhibition was enhanced 10-fold in cells pretreated with PM-19 compared with cells treated only after infection (Fig. 2D).

These memory effects of PM-19 were also evaluated by quantitation of cellular virus DNA using PCR.

After pretreatment at 10 μg ml^{-1} for 1 h prior to infection, PM-19 at 100 μg ml^{-1} was added to cells during the attachment or penetration stage of infection. The quantity of intracellular virus DNA was measured at 13 h after infection (before the second infection) using PCR. No memory effect of PM-19 on the attachment of virus to cells was detected. However, the quantity of intracellular virus DNA in cells treated with PM-19 during the penetration

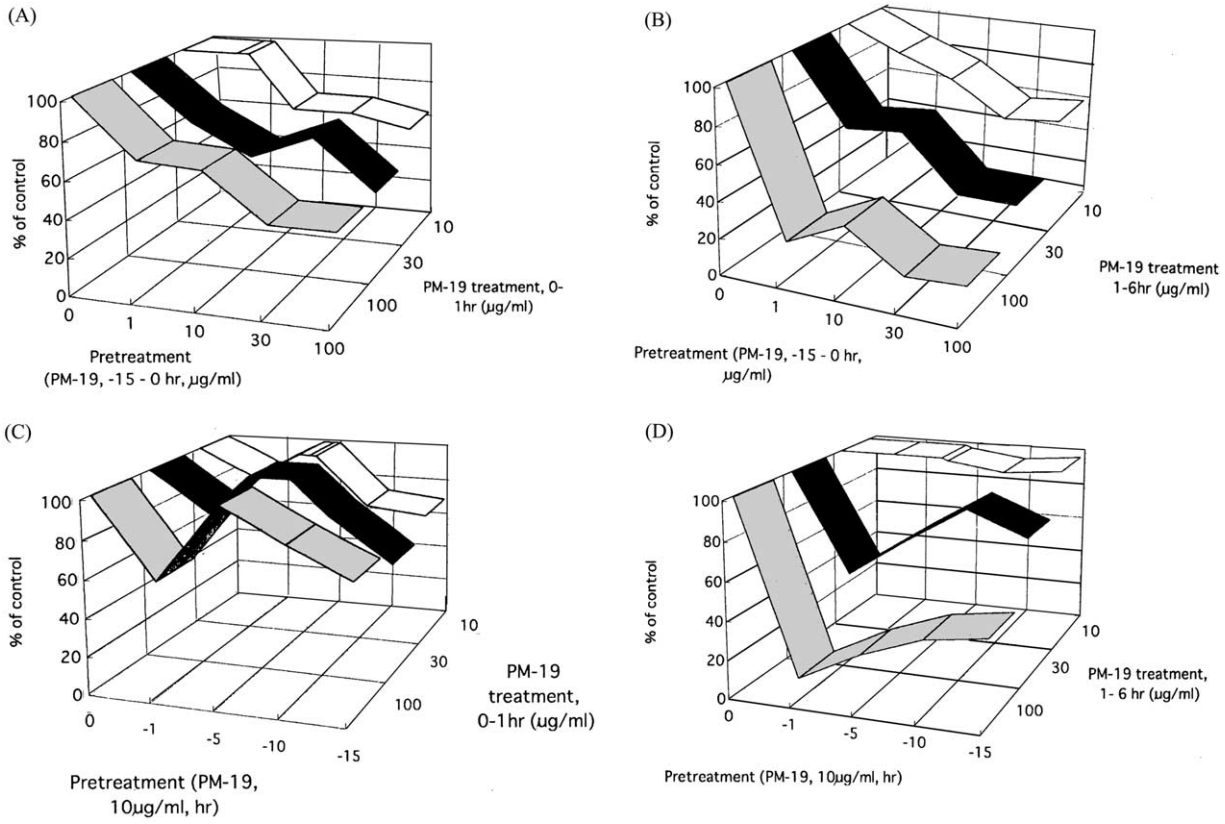

Fig. 2. (A–D) The memory effect of PM-19 pretreatment on HSV-2 attachment/penetration. PM-19-pretreated and nontreated Vero cells were infected with HSV-2 at 100 PFU per well and then were re-treated with various doses of PM-19 during the attachment or penetration stage. The memory effects were expressed as % of control plaque numbers. (A and B) Various doses of PM-19 during pretreatment from −15 to 0 h; (C and D) various time points of pretreatment with PM-19 (10 μg ml^{-1}). (A and C) PM-19 re-addition during the HSV-2 attachment stage; (B and D) PM-19 re-addition during the HSV-2 penetration stage. White strip: 10 μg ml^{-1} of PM-19 (re-addition); black strip: 30 μg ml^{-1}; gray strip: 100 μg ml^{-1}.

Table I
Memory effect of PM-19 (estimation of cellular HSV-2 DNA quantity)

Treatment	*Pretreatment*[a]	*Relative yield of HSV-2DNA (n:2)*	*Percent yield versus virus control*	*Percent yield versus pretreatment (−)*
Virus control	(−)	0.566		
	(+)	0.621		101
PM-19, 0–1 h[b]	(−)	0.554	98	
	(+)	0.628	101	113
PM-19, 1–13 h[b]	(−)	0.390	69	
	(+)	0.240	39	55

[a] PM-19, 10 μg ml^{-1}, −1 to 0 h. [b] PM-19, 100 μg ml^{-1}, time 0 is time of infection.

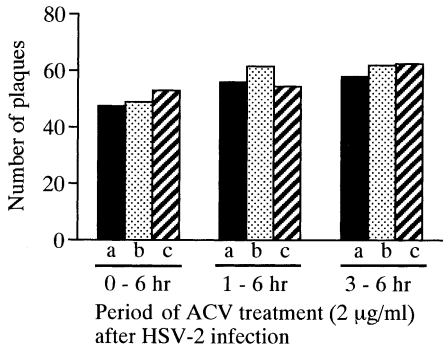

Fig. 3. The memory effect of ACV pretreatment on HSV-2 replication. ACV-pretreated and nontreated Vero cells were infected with HSV-2 at 100 PFU per well and then re-treated with ACV at 2 μg ml^{-1} to determine the effect on replication of HSV-2. Results were expressed as the number of plaques. (a) Nontreated, (b) −15 to 0 h, (c) −1 to 0 h of ACV treatment (2 μg ml^{-1}) prior to HSV-2 infection.

stage and thereafter was decreased to 55% of the quantity in pretreated cells (Table I).

On the other hand, the number of plaques formed on cells treated with ACV at 2 μg ml^{-1} for various periods after infection increased gradually with the delay of ACV addition. No memory effect of ACV pretreatment was seen (Fig. 3).

Memory effect of PM-19 on a second round of HSV-2 infection

Using the infectious center assay method, it was possible to determine the effect of PM-19 on a second round of HSV-2 infection. The second round of infection resulted in about a 70% virus yield, when PM-19 was present during the penetration stage, compared with the virus control of nontreated Vero cells. The yield was further reduced to 40% by PM-19 pretreatment prior to infection (Table II).

Table II
Memory effect of PM-19 (estimation of infectious centers)

Treatment			*Sample dilution*	*Number of infectious centers*				*Percent yield*		
Pretreatment[a]	*Treatment after infection*	*PEG*[b]		*1*	*2*	*Mean*	*log (PFU per well)*	*Virus alone*	*Pre, +/−*	*PEG, +/−*
Determination of second infection										
(−)	Virus alone	(−)	10^5	2	2	2.0	5.06			
			10^4	9	14	11.5				
(+)		(−)	10^5	1	1	1.0	5.04		95.5	
			10^4	10	12	11.0				
			10^3	53	59	56.0				
(−)	PM-19, 100 μg ml^{-1}; PI, 1–6 h	(−)	10^5	3	5	4.0	4.93	74.1		
			10^4	10	7	8.5				
(+)		(−)	10^5	1	0	0.5	4.64	39.8	51.3	
			10^4	6	9	7.5				
			10^3	29	24	26.5				
Determination of virus to cell fusion										
(−)	Virus alone	(+)	10^5	1	2	1.5	5.23			147.9
			10^4	16	18	17.0				
(+)		(+)	10^5	1	2	1.5	5.27		109.6	169.8
			10^4	19	18	18.5				
(−)	PM-19, 100 μg ml^{-1}; PI, 1–6 h	(+)	10^5	2	1	1.5	5.08	70.8		141.1
			10^4	10	14	12.0				
(+)		(+)	10^5	1	2	1.5	4.81	34.7	53.7	147.9
			10^4	8	5	6.5				

[a] PM-19 pretreatment (10 μg ml^{-1}, −1 to 0 h), time 0 is time of infection. [b] Polyethyleneglycol treatment to induce fusion.

Memory effect of PM-19 on the fusion stage of HSV-2 infection

The memory effect of PM-19 on the fusion of virions to cells was determined using the infectious center assay method. PM-19 treatment during the penetration of cells after infection decreased the number of infectious centers more potently in cells pretreated with PM-19. Thus, the memory effect of PM-19 pretreatment on virus penetration was maintained under the conditions of fusion induced by polyethyleneglycol treatment. The polyethyleneglycol-induced fusion increased the number of infectious centers in each treatment group by about 1.5-fold (Table II).

DISCUSSION

The keggin heteropolyoxotungstate PM-19 has been shown to be a potent PM inhibitor of the replication of HSV at a noncytotoxic concentration [6]. PM-19 mainly inhibits the replication of HSV-2 at the penetration stage [15]. It was also shown that the PM-19 added could be removed by washing the treated cells with PBS prior to the extraction of DNA. In this study, to elucidate whether PM-19-treated Vero cells had an altered response to HSV-2 infection, we studied the effect of PM-19 treatment prior to the infection.

The amount of PM-19 could be reduced to an ineffective level by washing the cells with PBS before infection (Fig. 1B, panel a). The pretreatment effect of PM-19 could not be detected on readdition of PM-19 from 0 to 72 h after infection. However, the effect could be detected by examining the attachment of virus and penetration of cells (Fig. 1B, panel b and c). The enhancement of the inhibition of HSV-2 plaque formation by PM-19 in cells pretreated with PM-19 was named the memory effect. The memory effect of PM-19 was greatest at especially on the penetration stage of HSV-2 infection, with a maximum 10-fold enhancement of the inhibition (Fig. 2D). Although the partial memory effect was detected during the attachment stage, as assayed by plaque formation, almost no effect was found in terms of measurement of intracellular virus DNA(Table I). These phenomenon suggest that PM-19 caused the retention of cell-attached or fused virions at the cell surface. These virions at the cell surface may be detected as intracellular virus DNA with a quantitative PCR method. On the other hand, no memory effect of ACV was detected (Fig. 3).

Pretreatment effects of some other antiviral agents have been reported. Among these, saikosaponon d was investigated for both its inactivating effects on some viruses and its antiviral effects against the viruses *in vitro* [20].

Pretreatment of cells with 9-(2-phosphonylmethoxyethyl)adenine (PMEA) before infection enhanced the antiviral potency by almost 10-fold compared with treatment of the cells only after viral infection [21]. These effects were elicited by PMEA metabolites [22]. On the other hand, a pretreatment effect of PM-19 could not be detected by the plaque assay or the quantitation of intracellular virus DNA and PM-19 have not activity of direct inactivation against the HSV virions [15]. Furthermore, the antiviral effect of PM-19 was not elicited by its metabolites, and the residual PM-19 could be reduced to an ineffective level by washing cells with PBS [15]. We showed that PM-19 mainly inhibits HSV-2 infection at the penetration stage, and we infer that almost no PM-19 is able to enter the cells.

Recently, some cellular molecules were reported to act as cofactor at the step of viral entry [23–27]. We speculate that PM-19 pretreatment may influence these cofactor necessary for the penetration by HSV-2 of the cells and PM-19-pretreated Vero cells may become much more resistant to viral infection by signals derived from the cofactor at the step of viral entry. Furthermore, the memory effect of PM-19 pretreatment on the penetration of cells was maintained under conditions of fusion induced by PEG (Table II). This result shows that PM-19 does not act at the fusion stage of HSV-2 infection. Using the infectious center assay method, the effect of PM-19 on a second round of HSV-2 infection was detected. The results suggest that PM-19 may be a new type of antiviral agent.

REFERENCES

1. Yamase T, Fujita H, Fukushima K. Medical chemistry of polyoxometalates. Part 1. Potent antitumor activity of polyoxomolybdates on animal transplantable tumors and human cancer xenograft. *Inorg Chem Acta* 1988; **151:** 15–8.
2. Fukuma M, Fukushima K, Seto Y, Fujita H. New type of antiviral agents—polyoxometalates. *Jpn J Pharmacol* 1989; **49(Suppl):** 72.
3. Dan K, Seto Y, Fujita H. Effect of new antiviral agent, PM-19, on virus induced diabetes. *Jpn J Pharmacol* 1990; **52(Suppl):** 168.
4. Hill CL, Weeks MS, Schinazi RF. Anti-HIV-1 activity, toxicity, and stability studies of representative structural families of polyoxometalates. *J Med Chem* 1990; **33:** 2767–72.
5. Inouye Y, Take Y, Tokutake Y, Yoshida T, Yamamoto A, Yamase T, Nakamura S. Inhibition of replication of human immunodeficiency virus by a heteropolyoxotungstate (PM-19). *Chem Pharm Bull* 1990; **38:** 285–7.
6. Fukuma M, Seto Y, Yamase T. *In vitro* antiviral activity of polyoxotungstate (PM-19) and other polyoxometalates against herpes simplex virus. *Antiviral Res* 1991; **16:** 327–39.
7. Inouye Y, Take Y, Tokutake Y, Yoshida T, Yamamoto A, Yamase T, Nakamura S. Antiviral activity of polyoxomolybdoeuropate PM-104 against human immunodeficiency virus type 1. *Chem Pharm Bull* 1991; **39:** 1638–40.
8. Take Y, Tokutake Y, Inouye Y, Yoshida T, Yamamoto A, Yamase T, Nakamura S. Inhibition of proliferation of human immunodeficiency virus type 1 by novel heteropolyoxotungstates *in vitro*. *Antiviral Res* 1991; **15:** 113–24.
9. Yamase T, Tomita K, Seto Y, Fujita H. Antitumor and antiviral activities of certain polyoxometalates. *Biomed Pharm Appl* 1991; **13:** 187–212.
10. Fujita H, Fujita T, Sakurai T, Yamase T, Seto Y. Antitumor activity of new antitumor substance, polyoxomolybdate, against several human cancers in athymic nude mice. *Tohoku J Exp Med* 1992; **168:** 421–6.
11. Yamamoto N, Schols D, Clercq ED, Debyser Z, Pauwels R, Balzarini J, Nakashima H, Baba M, Hosoya M, Snoeck R, Neyts J, Andrei G, Murrer BA, Theobald B, Bossard G, Henson G, Abrams M, Picker D. Mechanism of anti-human immunodeficiency virus action of polyoxometalates, a class of broad-spectrum antiviral agents. *Mol Pharmacol* 1992; **42:** 1109–17.

12. Inouye Y, Tokutake Y, Yoshida T, Seto Y, Fujita H, Dan K, Yamamoto A, Nishiya S, Yamase T, Nakamura S. *In vitro* antiviral activity of polyoxomolybdates. Mechanism of inhibitory effect of PM-104 $(NH_4)12H_2(Eu_4(MoO_4)(H_2O)_{16}(Mo_7O_{24})_4)\cdot 13H_2O$ on human immunodeficiency virus type 1. *Antiviral Res* 1993; **20:** 317–31.
13. Shigeta S, Mori S, Watanabe J, Soeda S, Takahashi K, Yamase T. Synergistic anti-influenza virus A (H1N1) activities of PM-523 (polyoxometalate) and ribavirin *in vitro* and *in vivo*. *Antimicrob Agents Chemother* 1997; **41:** 1423–7.
14. Dan K, Miyashita K, Seto Y, Yamase T. Quantitation of herpes simplex viral DNA in Vero cells for evaluation of an antiviral agent using the polymerase chain reaction. *J Virol Methods* 1998; **76:** 73–9.
15. Dan K, Miyashita K, Seto Y, Fujita H, Yamase T. Mechanism of the protective effect of heteropoly-oxotungstate (PM-19) against herpes simplex virus: discrimination between antiviral effects at each stage of viral replication by quantitative PCR. *Pharmacology* (in press).
16. Yamase T, Watanabe R. Photoredox chemistry of keggin dodecatungstoborate $[BW_{12}O_{40}]^{5-}$ and role of heterogeneous catalysis in hydrogen formation. *J Chem Soc Dalton Trans* 1986; 1669–75.
17. Wagstaff AJ, Faulds D, Goa KL. Acyclovir: a reappraisal of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. *Drugs* 1994; **47:** 153.
18. Kessler HH, Pierer K, Weber B, Sakrauski A, Santner B, Stuenzener D, Gergely E, Marth E. Detection of herpes simplex virus DNA from cerebrospinal fluid by PCR and a rapid, nonradioactive hybridization technique. *J Clin Microbiol* 1994; **32:** 1881–6.
19. Sarmiento M, Haffet M, Spear PG. Membrane proteins specified by herpes simplex viruses. III. Role of glycoprotein VP7(B2) in virion infectivity. *J Virol* 1979; **29:** 1149–58.
20. Ushio Y, Abe H. Inactivation of measles virus and herpes simplex virus by saikosaponin d. *Planta Med* 1992; **58:** 171–3.
21. Cerny J, Foster SA, Cheng Y. Cell-protecting effect against herpes simplex virus-1 and cellular metabolism of 9-(2-phosphonylmethoxyethyl)adenine in hela S3 cells. *Mol Pharmacol* 1992; **42:** 537–44.
22. Aduma P, Connelly MC, Srinivas RV, Fridland A. Metabolic diversity and antiviral activities of acyclic nucleoside phosphonates. *Mol Pharmacol* 1995; **47:** 816–22.
23. Montgomery RI, Warner MS, Lum BJ, Spear PG. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* 1996; **87(3):** 427–36.
24. Whitbeck JC, Peng C, Lou H, Xu R, Willis SH, Ponce de Leon M, Peng T, Nicola AV, Montgomery RI, Warner MS, Soulika AM, Spruce LA, Moore WT, Lambris JD, Spear PG, Cohen GH, Eisenberg RJ. Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. *J Virol* 1997; **71(8):** 6083–93.
25. Warner MS, Geraghty RJ, Martinez WM, Montgomery RI, Whitbeck JC, Xu R, Eisenberg RJ, Cohen GH, Spear PG. A cell surface protein with herpesvirus entry activity (HveB) confers susceptibility to infection by mutants of herpes simplex virus type 1, herpes simplex virus type 2, and pseudorabies virus. *Virology* 1998; **246:** 179–89.
26. Connolly SA, Whitbeck JC, Rux AH, Krummenacher C, van Drunen Little-van den Hurk S, Cohen GH, Eisenberg RJ. Glycoprotein D homologs in herpes simplex virus type 1, pseudorabies virus, and bovine herpes virus type 1 bind directly to human HveC (nectin-1) with different affinities. *Virology* 2001; **280(1):** 7–18.
27. Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD, Spear PG. A novel role for 3-*O*-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* 1999; **99(1):** 13–22.